

Contents lists available at ScienceDirect

### Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca





# Composition and bioaccessibility of inorganic elements in plant-based yogurts

Ana Paula Rebellato <sup>a, \*</sup>, Maria Isabel Andrekowisk Fioravanti <sup>b</sup>, Raquel Fernanda Milani <sup>a</sup>, Marcelo Antônio Morgano <sup>a</sup>

- a Institute of Food Technology, Av. Brasil 2880, Jd. Chapadão, P.O. Box 139, Campinas, SP 13070-178, Brazil
- <sup>b</sup> Adolfo Lutz Institute, Rua São Carlos, 720, Vila Industrial, Campinas, SP 13035-420, Brazil

#### ARTICLE INFO

Keywords: Minerals Trace elements in vitro digestion INFOGEST 2.0 protocol Food safety

#### ABSTRACT

This study aimed to evaluate the composition of inorganic elements as well as their bioaccessibility in commercial plant-based yogurt. The samples were mineralized using fast ultrasound-assisted acid digestion, and the inorganic elements were determined by ICP-MS. The method was validated according to the INMETRO guide, with recoveries from 99% to 111%; precision from 2% to 14%, and LOQ ranging from 0.4  $\mu$ g/100 g to 2.5 mg/ 100 g. The element concentrations (mg/100 g) of the plant-based yogurts were Ca(<LOQ-239.90); Cu(<LOQ-0.16); Fe (0.07–1.48); K(27.0–226.4); Mg(3.72–23.9); Mn(0.05–0.34); Na(2.77–165.3); P(7.80–189.6); Zn (<LOQ-1.06), and Se (<LOQ-2.18  $\mu$ g/100 g). In turn, the animal-based yogurts contained only Ca, K, Mg, Na, Zn, and Se. The bioaccessible Ca was higher in animal-based yogurt (41%), while the bioaccessible fraction of Mn (30%), Cu(25–61%), and Fe(9–33%) was only quantified in the plant-based yogurts. Regarding the trace elements, some samples showed bioaccessibility above 50% for Cr, Ni, Mo, Ba, and Co, while the animal-based yogurts showed bioaccessibility was similar to that of animal-based yogurt. The bioaccessible fraction (>50%) demonstrates the importance of knowing the composition of plant-based foods to ensure the safety and health of consumers despite the lower contents of the trace elements.

### 1. Introduction

The plant-based food market is growing and consumers (vegetarians, vegans, flexitarians, and the general population) are increasingly seeking plant-based alternatives to animal products. The impact of this demand has led food companies to invest in the development of new non-dairy products (Mäkinen et al., 2016) to provide tasty foods that can meet their nutritional needs and have functional properties, such as the presence of fiber, vitamins, and minerals, considered to be health promoters (Bernat et al., 2014; Sethi et al., 2016; Singhal et al., 2017).

Although plant-based beverages are well-established and widely consumed, novel products such as plant-based yogurts are also emerging. Unlike fermented milk yogurts, plant-based yogurts do not present concerns related to lactose intolerance and cholesterol (Lomer et al., 2007; Tangyu et al., 2019). However, studies have shown that plant beverages have a lower nutritional value when compared to their bovine analog, especially concerning protein and mineral contents.

Thus, they are usually fortified with calcium to increase their nutritional quality and make them viable substitutes for animal products (Jeske et al., 2017; Mäkinen et al., 2016; Sethi et al., 2016). Despite the healthy appeal and increased consumption, there is a variation in their nutritional composition, requiring further studies, mainly regarding the bioavailability of nutrients to be used as substitutes for animal products (Singhal et al., 2017).

Depending on the composition of the raw material used (oilseeds, legumes, cereals, fruits, rice, among others), bioactive compounds, fibers, vitamins, and minerals such as phosphorus, potassium, zinc, manganese, copper, selenium, calcium, magnesium, and iron may be present in the final product, thus contributing to human health (Bernat et al., 2014; Felberg et al., 2009; Sethi et al., 2016; Singhal et al., 2017).

Minerals are indispensable for organic functions, both in ionic form and as constituents of compounds such as hormones, enzymes, and tissue proteins, making up about 4% of total body weight (Xia et al., 2017). On the other hand, toxic elements can cause damage to various organs

E-mail address: paularebe@hotmail.com (A.P. Rebellato).

<sup>\*</sup> Corresponding author.

after they leave the systemic circulation. However, the accumulation over time is a worrying topic, leading to damage generated by mechanisms such as the breakdown of enzymes, hormones, proteins, and cell membranes, among others, once they have no biological function (Patwa et al., 2022; Patwa & Flora, 2020).

Studies in the literature have reported that the essential minerals in different matrices such as rice, almonds, soluble soybean extract, and coconut can vary from 4 to 237 mg/100 g for the element Ca, from 2 to 222 mg/100 g for Mg, from 0.15 to 1.95 mg/100 g for Mn, from trace value to 3.1 mg/100 g for Fe, from 0.02 to 0.93 mg/100 g for Cu, and from 0.3 to 2.6 mg/100 g for Zn (TACO, 2011). However, like essential minerals, toxic elements can be retained during processing and in the final product. Thus, the safety of plant-based foods depends not only on the characteristics of the raw materials used, but also on external factors such as soil, inputs, harvest, storage, transport, processing, and post-processing handling. Thus, monitoring the levels of inorganic elements considered essential and toxic, and their bioaccessibility, are necessary for this type of food (Codina-Torrella et al., 2017; Fioravanti et al., 2023; Jeske et al., 2017; Singhal et al., 2017).

In Brazil, no regulation defines the quality parameters of plant-based (PB) products. Few studies have been discussed about the implementation of ISO 23662, which defines and establishes criteria for the entire production chain of vegan and vegetarian foods, in addition to labeling parameters and label claims. In June 2021, Ordinance. 327 was published, inviting the public to participate in the Public Consultation to obtain information that may assist in the regulation of processed products of plant origin to replace animal-based products, self-denominated as plant-based. Recently, the European Commission updated the technical regulation (EU - n° 2023/915) on maximum levels contaminants in foodstuffs and information about plant-based products was not added (European Commission, 2023,2023). Regarding the provision of information to consumers on foodstuffs, both the Food and Drug Administration (FDA), through the Code of Federal Regulations, and the European Parliament (EU) do not establish thresholds for inorganic contaminants in plant-based products (CFR, 2023; European Commission, 2023). In nutrition-related studies, it is of paramount importance to consider both the risk or benefit assessment associated with the intake of a particular element. Moreover, not only the determination of total levels must be considered since not every substance ingested is absorbed and effectively utilized by the human body. Thus, methods in vitro have been proposed for the determination of the bioaccessibility of compounds present in food, once they are faster, simpler, relatively low cost, and are performed under controlled conditions (Laparra et al., 2003; Minekus et al., 2014). In vitro assays can simulate, in a simplified way, physiological conditions such as temperature, the chemical composition of the digestive fluids, pH, and residence times in each compartment (Fernandez-Garcia et al., 2009; Minekus et al., 2014). Most models that simulate gastrointestinal digestion are static, as they simulate the digestive transit by sequential (compartmental) exposure of food under simulated conditions from the stomach and small intestine, or from the mouth, stomach, and small intestine (Cardoso et al., 2015; Minekus et al., 2014; Thakur et al., 2020).

As the consumption of plant-based products is constantly increasing, mainly as an alternative to cow's milk, it is critical to study both the total content and the bioaccessible fraction of inorganic elements. The composition of the essential and potentially toxic elements may vary depending on the raw material, the industrial processes, the use of fortifiers, the presence of anti-nutritional factors, among others (Part et al., 2023; Silva et al., 2020). Silva et al. (2020) verified that the content and bioaccessibility of essential minerals varied widely; samples of plant-based beverages not fortified with Ca presented less amount of Ca content than cow's milk, however samples that were fortified presented comparable values for total Ca and more Ca bioaccessibility than cow's milk.

Therefore, the objective of this study was to evaluate the composition of essential and trace elements such as aluminum (Al), barium (Ba),

**Table 1**ICP OES and ICP-MS parameters for the determination of inorganic contaminants in yogurt samples.

ICP OES conditions	
Power (RF)	1200 W
Air flow rate/ Auxiliary air	12.0 / 1.0 L min <sup>-1</sup>
Nebulization Chamber	Double-pass cyclonic
Nebulizer flow rate	Seaspray; 0.70 L min <sup>-1</sup>
Wavelength (nm)	Ca (317.933), Cu (324.754), Fe (259.940), P (213.618), Mg
	(279.553), Mn (257.610), K (766.491), Na (589.592), Zn
	(206.200)
ICP-MS conditions	
Power (RF)	1550 W
Air flow rate/	14.0 / 0.8 L min <sup>-1</sup>
Auxiliary air	
He Flow rate	$5.00 \mathrm{~mL~min^{-1}}$
Nebulizer flow rate	Micromist; 0.98 L min <sup>-1</sup>
Nebulization	Double-pass cyclonic at 2.8°C
Chamber	
Dwell Time	0.3 s / 0.02 s (PI)
Monitored Isotopes	<sup>27</sup> Al, <sup>53</sup> Cr, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>78</sup> Se, <sup>97</sup> Mo, <sup>137</sup> Ba
Internal standard	<sup>45</sup> Sc; <sup>72</sup> Ge; <sup>115</sup> In; <sup>103</sup> Rh; <sup>209</sup> Bi; <sup>195</sup> Pt
( <b>IS):</b> 50 μg/L	

calcium (Ca), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), sodium (Na), phosphorus (P), zinc (Zn), and Se (selenium) in plant-based yogurts and to determine the bioaccessible fraction of inorganic elements, aimed to ensure the quality and food safety of plant-based products for the population.

### 2. Materials and methods

### 2.1. Reagents and solutions

Analytical grade reagents were used in the experiments: water purified in a reverse osmosis system with a resistivity less than  $18.2\,\mathrm{M}\,\Omega$  cm (Gehaka, São Paulo, Brazil); concentrated nitric acid purified by subboiling distillation (Distillacid, Berghof, Eningen, Germany); 37% hydrochloric acid, 30% hydrogen peroxide (H2O2) (v/v) (Merck, Darmstadt, Germany),  $\alpha$ -amylase (10080), porcine pepsin (P6887), bile bovine (B3883), pancreatin (P7545) from Sigma Aldrich (St. Louis, EUA) and other reagents used for the preparation of salivary, gastric, and enteric fluids, as specified in the INFOGEST protocol (Brodkorb et al., 2019).

Certified reference material (CRM): SRM 1547 Peach leaves (National Institute of Standards and Technology, Gaithersburg, EUA) and ERM-BD 151 skimmed milk powder (Joint Research Center, Geel, Bélgica), standard solutions of 100 or 1000 mg/L (Specsol-Quimlab, Jacareí, Brazil) containing the minerals Ca, Cu, Fe, K, Mg, Mn, Na, P, Zn, and Se, and the contaminants Al, Ba, Cr, Co, Mo, and Ni were also used.

### 2.2. Apparatus

An ultrasonic bath (Easy 180 H, Elma, Germany) was used for the acid digestion of the samples. The elements Ca, Cu, Fe, P, Mg, Mn, K, Na, and Zn were determined by inductively coupled plasma optical emission spectrometry (ICP OES) (5100 VDV, Agilent Technologies, Tokyo, Japan), while the elements Al, Cr, Co, Ni, Se, Mo, and Ba were determined by inductively coupled plasma mass spectrometry (ICP-MS) (iCAP RQ, Thermo Scientific, Germany). The operating conditions are described in Table 1.

### 2.3. Sampling

From August to October 2022, 44 samples of plant-based yogurt and

 Table 2

 Concentration of essential inorganic elements of plant-based and animal-based yogurts (mean  $\pm$  SD).

Samples	Brands	Lot	Ca (mg/100 g)	Cu (mg/ 100 g)	Fe (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	Mn (mg/100 g)	Na (mg/100 g)	P (mg/100 g)	Zn (mg/100 g)	Se (μg/100 g)
1	VV	Α	$13.0\pm0.2^{\text{a}}$	$0.10 \pm 0.00^{a}$	$1.22 \pm 0.03^{a}$	$47.5 \pm 1.3^{a}$	$4.92 \pm 0.12^{a}$	$0.07 \pm 0.00^{a}$	59.7 ± 1.6 <sup>a</sup>	$51.4\pm1.3^{\text{a}}$	$0.56 \pm 0.02^{\rm b}$	$17.6\pm2.1^{\mathrm{a}}$
	VV	В	$9.88 \pm 0.01^{b}$	$\begin{array}{l} 0.09 \pm \\ 0.00^{ab} \end{array}$	$\begin{array}{c} 1.12 \pm \\ 0.01^{\mathrm{b}} \end{array}$	$\begin{array}{l} \textbf{41.6} \pm \\ \textbf{0.1}^{\text{b}} \end{array}$	$\begin{array}{l} \textbf{4.21} \pm \\ \textbf{0.00}^{\text{b}} \end{array}$	$\begin{array}{l} \textbf{0.06} \pm \\ \textbf{0.00}^{\text{b}} \end{array}$	$\begin{array}{l} 54.8\ \pm \\ 0.1^b \end{array}$	$46.5\pm0.3^{b}$	$\begin{array}{l} \textbf{0.40} \pm \\ \textbf{0.02}^c \end{array}$	$11.9\pm0.1^{\rm b}$
	VV	С	$10.2\pm0.3^{\rm b}$	$\begin{array}{c} \textbf{0.09} \pm \\ \textbf{0.01}^{\text{b}} \end{array}$	$1.03 \pm 0.01^{\rm c}$	$\begin{array}{l} \textbf{43.3} \pm \\ \textbf{0.8}^{\text{b}} \end{array}$	$\begin{array}{l} 4.24 \; \pm \\ 0.09^{b} \end{array}$	$0.06 \pm 0.00^{\rm b}$	$\begin{array}{l} 53.8 \; \pm \\ 0.9^b \end{array}$	$45.8 \pm 0.7^{\mathrm{b}}$	$\begin{array}{c} 1.06 \pm \\ 0.00^a \end{array}$	$20.0\pm1.8^{a}$
2	VV	A	$10.7\pm0.3^{\rm b}$	$0.10 \pm 0.00^{a}$	$^{1.02~\pm}_{0.02^{ m b}}$	$\begin{array}{l} \textbf{46.1} \pm\\ \textbf{0.6}^{\text{b}} \end{array}$	$\begin{array}{l} 4.59 \pm \\ 0.08^{\mathrm{b}} \end{array}$	$0.06 \pm 0.00^{\rm b}$	$51.9 \pm 0.9^{b}$	$43.6\pm0.8^{b}$	$\begin{array}{l} \textbf{0.45} \pm \\ \textbf{0.00}^{\text{b}} \end{array}$	$14.0 \pm 0.4^{b}$
	VV	В	$13.4 \pm 0.2^{a}$	$0.11 \pm 0.01^{a}$	$1.50 \pm 0.03^{a}$	$58.7 \pm 0.6^{a}$	$5.68 \pm 0.06^{a}$	$0.08 \pm 0.00^{a}$	$73.5 \pm 0.8^{a}$	$56.9 \pm 0.6^{a}$	$\begin{array}{l} 0.72 \pm \\ 0.04^a \end{array}$	$13.2 \pm 0.6^{\mathrm{b}}$
	VV	C	$10.0 \pm 0.1^{c}$	$0.09 \pm 0.00^{b}$	$0.93 \pm 0.02^{c}$	44.9 ± 0.5 <sup>b</sup>	$4.15 \pm 0.04^{c}$	$0.06 \pm 0.00^{\rm b}$	52.7 ± 0.5 <sup>b</sup>	$43.1 \pm 0.4^{b}$	0.51 ± 0.04 <sup>b</sup>	$20.7 \pm 0.7^{a}$
3	VV	A	$12.5 \pm 0.3^{a}$	$0.12 \pm 0.01^{a}$	$1.42 \pm 0.06^{a}$	55.5 ± 1.5 <sup>a</sup>	5.64 ± 0.13 <sup>a</sup>	$0.07 \pm 0.00^{a}$	$70.1 \pm 1.9^{a}$	$54.8 \pm 1.3^{a}$	$0.72 \pm 0.03^{a}$	$13.2 \pm 2.3^{\rm b}$ $13.3 \pm 0.2^{\rm b}$
	VV VV	B C	$12.2 \pm 0.2^{a}$ $10.3 \pm 0.3^{b}$	$\begin{array}{l} 0.10 \; \pm \\ 0.00^{ab} \\ 0.09 \; \pm \end{array}$	$1.39 \pm 0.03^{a} \ 0.97 \pm$	$53.8 \pm 0.8^{a} \ 43.5 \pm$	$5.40 \pm 0.07^{a} \ 4.25 \pm$	$0.07 \pm 0.00^{a} \ 0.06 \pm$	$70.8 \pm 1.2^{a} \\ 53.9 \pm$	$54.5 \pm 0.8^{a}$ $44.1 \pm 1.1^{b}$	$0.36 \pm 0.03^{c} \\ 0.47 \pm$	$13.3 \pm 0.2$ $19.8 \pm 0.3^{a}$
4	VV	A	10.3 ± 0.3	0.09 ± 0.01 <sup>c</sup> <loq< td=""><td>0.97 ± 0.04<sup>b</sup> 0.16 ±</td><td>1.3<sup>b</sup> 45.8 ±</td><td>4.25 ± 0.14<sup>b</sup> 5.20 ±</td><td>0.00 ± 0.00<sup>b</sup> 0.07 ±</td><td>1.7<sup>b</sup> 9.05 ±</td><td>79.3 <math>\pm</math> 1.2<sup>b</sup></td><td>0.47 ± 0.02<sup>b</sup> 0.24 ±</td><td>19.8 ± 0.3</td></loq<>	0.97 ± 0.04 <sup>b</sup> 0.16 ±	1.3 <sup>b</sup> 45.8 ±	4.25 ± 0.14 <sup>b</sup> 5.20 ±	0.00 ± 0.00 <sup>b</sup> 0.07 ±	1.7 <sup>b</sup> 9.05 ±	79.3 $\pm$ 1.2 <sup>b</sup>	0.47 ± 0.02 <sup>b</sup> 0.24 ±	19.8 ± 0.3
4	VV	В	$204 \pm 2^{b}$	<loq <loq< td=""><td>0.10 ± 0.00<sup>b</sup> 0.13 ±</td><td>0.8<sup>a</sup> 34.8 ±</td><td>0.10<sup>a</sup> 4.09 ±</td><td>0.00 ± 0.00<sup>a</sup> 0.05 ±</td><td>9.03 ± 0.36<sup>a</sup> 9.52 ±</td><td><math>79.3 \pm 1.2</math> <math>85.7 \pm 0.8^{a}</math></td><td>0.24 ± 0.02<sup>b</sup> <loq< td=""><td><loq <loq< td=""></loq<></loq </td></loq<></td></loq<></loq 	0.10 ± 0.00 <sup>b</sup> 0.13 ±	0.8 <sup>a</sup> 34.8 ±	0.10 <sup>a</sup> 4.09 ±	0.00 ± 0.00 <sup>a</sup> 0.05 ±	9.03 ± 0.36 <sup>a</sup> 9.52 ±	$79.3 \pm 1.2$ $85.7 \pm 0.8^{a}$	0.24 ± 0.02 <sup>b</sup> <loq< td=""><td><loq <loq< td=""></loq<></loq </td></loq<>	<loq <loq< td=""></loq<></loq 
	VV	C	$204 \pm 2$ $210 \pm 3^{a}$	<loq <loq< td=""><td><math>0.13 \pm 0.01^{c} \\ 0.20 \pm</math></td><td>0.3<sup>c</sup> 39.1 ±</td><td>0.04<sup>c</sup> 4.87 ±</td><td>0.00<sup>b</sup> 0.05 ±</td><td>0.30<sup>a</sup> 9.34 ±</td><td><math>87.4 \pm 1.3^{a}</math></td><td>0.45 ±</td><td><loq< td=""></loq<></td></loq<></loq 	$0.13 \pm 0.01^{c} \\ 0.20 \pm$	0.3 <sup>c</sup> 39.1 ±	0.04 <sup>c</sup> 4.87 ±	0.00 <sup>b</sup> 0.05 ±	0.30 <sup>a</sup> 9.34 ±	$87.4 \pm 1.3^{a}$	0.45 ±	<loq< td=""></loq<>
5	VV	A	$165 \pm 1^{\mathrm{b}}$	<loq <loq< td=""><td>0.20 ± 0.01<sup>a</sup> 0.07 ±</td><td>0.6<sup>b</sup> 33.0 ±</td><td>0.04<sup>b</sup> 3.87 ±</td><td>0.00<sup>b</sup> 0.05 ±</td><td>0.13<sup>a</sup> 7.60 ±</td><td><math>69.8 \pm 0.5^{\mathrm{b}}</math></td><td>0.45<sup>a</sup> <loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<></loq 	0.20 ± 0.01 <sup>a</sup> 0.07 ±	0.6 <sup>b</sup> 33.0 ±	0.04 <sup>b</sup> 3.87 ±	0.00 <sup>b</sup> 0.05 ±	0.13 <sup>a</sup> 7.60 ±	$69.8 \pm 0.5^{\mathrm{b}}$	0.45 <sup>a</sup> <loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
J	vv	В	199 ± 1 <sup>a</sup>	<loq< td=""><td>0.01<sup>c</sup> 0.25 ±</td><td>0.4° 51.5 ±</td><td>0.03<sup>c</sup> 5.89 ±</td><td>0.00° 0.09 ±</td><td>0.08<sup>c</sup> 11.1 ±</td><td><math>87.3 \pm 4.9^{a}</math></td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	0.01 <sup>c</sup> 0.25 ±	0.4° 51.5 ±	0.03 <sup>c</sup> 5.89 ±	0.00° 0.09 ±	0.08 <sup>c</sup> 11.1 ±	$87.3 \pm 4.9^{a}$	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	VV	С	$200\pm3^a$	<loq< td=""><td><math display="block">\begin{array}{l} 0.03^a \\ 0.19 \ \pm \end{array}</math></td><td><math display="block">\begin{array}{l} 2.9^a \\ 40.6 \ \pm \end{array}</math></td><td><math>0.34^{a}</math> 4.79 <math>\pm</math></td><td><math display="block">\begin{array}{l} 0.01^a \\ 0.06 \ \pm \end{array}</math></td><td><math display="block">0.6^{a} \\ 10.1 \pm</math></td><td><math display="block">86.6\pm1.3^{\rm a}</math></td><td>0.12 ±</td><td><loq< td=""></loq<></td></loq<>	$\begin{array}{l} 0.03^a \\ 0.19 \ \pm \end{array}$	$\begin{array}{l} 2.9^a \\ 40.6 \ \pm \end{array}$	$0.34^{a}$ 4.79 $\pm$	$\begin{array}{l} 0.01^a \\ 0.06 \ \pm \end{array}$	$0.6^{a} \\ 10.1 \pm$	$86.6\pm1.3^{\rm a}$	0.12 ±	<loq< td=""></loq<>
	vv	D	$150\pm2^{c}$	<loq< td=""><td><math>0.00^{ m b} \ 0.29 \ \pm</math></td><td><math display="block">\begin{array}{l} 0.6^{\rm b} \\ 27.4 \ \pm \end{array}</math></td><td><math>0.07^{ m b} \ 3.72 \ \pm</math></td><td><math>0.00^{ m b} \ 0.06 \pm</math></td><td><math display="block">\begin{array}{l} 0.2^b \\ 4.29 \ \pm \end{array}</math></td><td><math display="block">71.5\pm0.9^{\text{b}}</math></td><td>0.00<sup>a</sup> <loq< td=""><td>4.15 <math>\pm</math></td></loq<></td></loq<>	$0.00^{ m b} \ 0.29 \ \pm$	$\begin{array}{l} 0.6^{\rm b} \\ 27.4 \ \pm \end{array}$	$0.07^{ m b} \ 3.72 \ \pm$	$0.00^{ m b} \ 0.06 \pm$	$\begin{array}{l} 0.2^b \\ 4.29 \ \pm \end{array}$	$71.5\pm0.9^{\text{b}}$	0.00 <sup>a</sup> <loq< td=""><td>4.15 <math>\pm</math></td></loq<>	4.15 $\pm$
6	VV	Α	$155\pm2^{b}$	<loq< td=""><td><math>0.01^{ m a} \ 0.09 \ \pm</math></td><td><math display="block">\begin{array}{l} 0.3^{\rm d} \\ 42.8 \ \pm \end{array}</math></td><td><math>0.05^{c}</math> 4.70 <math>\pm</math></td><td><math>0.00^{ m b} \ 0.07 \pm</math></td><td><math display="block">\begin{array}{l} 0.03^{\rm d} \\ 7.39 \ \pm \end{array}</math></td><td><math display="block">68.0 \pm 0.6^{\text{b}}</math></td><td><loq< td=""><td>0.59<sup>a</sup> <loq< td=""></loq<></td></loq<></td></loq<>	$0.01^{ m a} \ 0.09 \ \pm$	$\begin{array}{l} 0.3^{\rm d} \\ 42.8 \ \pm \end{array}$	$0.05^{c}$ 4.70 $\pm$	$0.00^{ m b} \ 0.07 \pm$	$\begin{array}{l} 0.03^{\rm d} \\ 7.39 \ \pm \end{array}$	$68.0 \pm 0.6^{\text{b}}$	<loq< td=""><td>0.59<sup>a</sup> <loq< td=""></loq<></td></loq<>	0.59 <sup>a</sup> <loq< td=""></loq<>
	vv	В	$204\pm 6^a$	<loq< td=""><td><math>0.01^{\rm c} \ 0.14 \pm</math></td><td><math>0.5^{ m b}</math> 54.9 <math>\pm</math></td><td><math>0.05^{\rm c} \\ 6.19 \pm</math></td><td><math display="block">\begin{array}{l} \textbf{0.00}^{\text{b}} \\ \textbf{0.08} \ \pm \end{array}</math></td><td><math>0.09^{\rm c}</math> 11.1 <math>\pm</math></td><td><math display="block">85.0\pm2.6^a</math></td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	$0.01^{\rm c} \ 0.14 \pm$	$0.5^{ m b}$ 54.9 $\pm$	$0.05^{\rm c} \\ 6.19 \pm$	$\begin{array}{l} \textbf{0.00}^{\text{b}} \\ \textbf{0.08} \ \pm \end{array}$	$0.09^{\rm c}$ 11.1 $\pm$	$85.0\pm2.6^a$	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	VV	С	$205\pm1^a$	<loq< td=""><td>0.00<sup>b</sup> 0.17 ±</td><td><math>1.6^{a}</math> 52.6 ±</td><td>0.17<sup>a</sup> 5.88 ±</td><td>0.00<sup>a</sup> 0.08 ±</td><td>0.3<sup>a</sup> 10.4 ±</td><td><math display="block">\textbf{87.8} \pm \textbf{0.4}^{a}</math></td><td><loq< td=""><td><loq< td=""></loq<></td></loq<></td></loq<>	0.00 <sup>b</sup> 0.17 ±	$1.6^{a}$ 52.6 ±	0.17 <sup>a</sup> 5.88 ±	0.00 <sup>a</sup> 0.08 ±	0.3 <sup>a</sup> 10.4 ±	$\textbf{87.8} \pm \textbf{0.4}^{a}$	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
7	vv	A	<loq< td=""><td>0.07 ±</td><td>0.01<sup>a</sup> 0.18 ±</td><td>0.2<sup>a</sup> 82.6 ±</td><td><math display="block">0.02^{b} \\ 10.4 \pm 0.1^{b}</math></td><td>0.00<sup>a</sup> 0.18 ±</td><td>0.1<sup>b</sup> 11.5 ±</td><td><math display="block">13.8 \pm 0.1^{b}</math></td><td>0.08 ±</td><td><loq< td=""></loq<></td></loq<>	0.07 ±	0.01 <sup>a</sup> 0.18 ±	0.2 <sup>a</sup> 82.6 ±	$0.02^{b} \\ 10.4 \pm 0.1^{b}$	0.00 <sup>a</sup> 0.18 ±	0.1 <sup>b</sup> 11.5 ±	$13.8 \pm 0.1^{b}$	0.08 ±	<loq< td=""></loq<>
	vv	В	<loq< td=""><td><math>0.00^{a} \ 0.07 \pm \ 0.00^{a}</math></td><td><math>0.01^{ m c} \ 0.25 \pm \ 0.01^{ m b}</math></td><td><math>0.5^{ m b} \\ 98.7 \pm \\ 3.4^{ m a}</math></td><td><math display="block">12.6\pm0.4^a</math></td><td><math>0.00^{ m b} \ 0.21 \pm \ 0.01^{ m a}</math></td><td><math>0.1^{ m c}\ 15.3~\pm\ 0.5^{ m a}</math></td><td><math display="block">15.5\pm0.5^a</math></td><td><math>0.01^{a} \ 0.06 \pm \ 0.01^{b}</math></td><td><loq< td=""></loq<></td></loq<>	$0.00^{a} \ 0.07 \pm \ 0.00^{a}$	$0.01^{ m c} \ 0.25 \pm \ 0.01^{ m b}$	$0.5^{ m b} \\ 98.7 \pm \\ 3.4^{ m a}$	$12.6\pm0.4^a$	$0.00^{ m b} \ 0.21 \pm \ 0.01^{ m a}$	$0.1^{ m c}\ 15.3~\pm\ 0.5^{ m a}$	$15.5\pm0.5^a$	$0.01^{a} \ 0.06 \pm \ 0.01^{b}$	<loq< td=""></loq<>
	vv	С	$\begin{array}{c} 1.93 \pm \\ 0.08^a \end{array}$	$0.00 \pm 0.00^{a}$	$0.01$ $0.29 \pm 0.01^{a}$	$100 \pm 10^{a}$	$12.6\pm0.1^{a}$	$0.01 \pm 0.00^{a}$	$13.4 \pm 0.2^{\rm b}$	$15.6\pm0.2^{a}$	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
8	vv	Α	$3.10 \pm 0.28^{a}$	$0.07 \pm 0.00^{ab}$	$0.01 \pm 0.01^{c}$	$\begin{array}{l} \textbf{87.4} \pm \\ \textbf{2.4}^{b} \end{array}$	$10.1\pm0.2^{b}$	$0.18 \pm 0.00^{ m b}$	$12.5 \pm 0.4^{ m b}$	$13.4 \pm 0.1^{b}$	$\begin{array}{l} 0.23 \pm \\ 0.02^{\mathrm{b}} \end{array}$	<loq< td=""></loq<>
	VV	В	2.56 ± 0.06 <sup>b</sup>	$0.08 \pm 0.01^{a}$	$0.26 \pm 0.02^{\rm b}$	$107\pm2^{\mathrm{a}}$	$12.4\pm0.3^a$	$0.21 \pm 0.01^{a}$	13.7 ± 0.3 <sup>a</sup>	$15.3\pm0.4^{a}$	<loq< td=""><td><loq< td=""></loq<></td></loq<>	<loq< td=""></loq<>
	VV	С	$2.50 \pm 0.09^{b}$	$0.06 \pm 0.00^{\rm b}$	$0.62 \pm 0.01^{a}$	$81.5 \pm 0.7^{\rm c}$	$9.00 \pm 0.08^{c}$	$0.17 \pm 0.00^{ m b}$	10.7 ± 0.1°	$11.8 \pm 0.2^{c}$	$\begin{array}{l} 0.56 \pm \\ 0.01^a \end{array}$	$5.04 \pm 0.64^{a}$
9	FR	Α	$15.6\pm1.0^a$	$\begin{array}{l} 0.07 \pm \\ 0.00^{\mathrm{b}} \end{array}$	$\begin{array}{l} 0.29 \pm \\ 0.02^a \end{array}$	$180\pm7^a$	$\begin{array}{l} 8.081  \pm \\ 0.24^{b} \end{array}$	$\begin{array}{l} \textbf{0.09} \pm \\ \textbf{0.00}^{\mathrm{b}} \end{array}$	$\begin{array}{l} 3.36~\pm\\ 0.17^{b} \end{array}$	$14.4 \pm 0.4^{b}$	$\begin{array}{l} 0.80 \pm \\ 0.16^a \end{array}$	$\begin{array}{l} \textbf{5.41} \pm\\ \textbf{0.42}^{\text{b}} \end{array}$
	FR	В	$12.8 \pm 0.4^{\text{b}}$	$0.09 \pm 0.00^{a}$	$0.26 \pm 0.03^{a}$	$191\pm3^a$	$\begin{array}{l} 8.92 \pm \\ 0.17^a \end{array}$	$0.09 \pm 0.00^{\rm b}$	$\begin{array}{l} 3.86 \pm \\ 0.03^a \end{array}$	$16.4\pm0.2^a$	$\begin{array}{l} \textbf{0.08} \pm \\ \textbf{0.01}^{b} \end{array}$	$\begin{array}{l} \textbf{4.45} \pm \\ \textbf{0.89}^{b} \end{array}$
	FR	С	$11.4\pm0.6^{b}$	$\begin{array}{l} 0.06 \pm \\ 0.00^{\mathrm{b}} \end{array}$	$\begin{array}{l} 0.24 \pm \\ 0.02^a \end{array}$	$167\pm1^{\rm b}$	$\begin{array}{l} \textbf{7.85} \pm \\ \textbf{0.04}^{b} \end{array}$	$0.10 \pm 0.00^{a}$	$\begin{array}{l} 3.27~\pm\\0.02^b\end{array}$	$14.0\pm0.1^{\rm b}$	$\begin{array}{l} 0.13 \pm \\ 0.01^b \end{array}$	$10.8\pm2.0^a$
10	FR	A	$6.44 \pm 0.27^{a}$	$\begin{array}{l} 0.05 \pm \\ 0.00^a \end{array}$	$0.28 \pm 0.03^{a}$	$60.4 \pm 0.7^{a}$	$\begin{array}{l} 5.81\ \pm \\ 0.08^a \end{array}$	$0.15 \pm 0.00^{a}$	$7.40 \pm 0.08^{a}$	$9.42 \pm 0.16^{a}$	$\begin{array}{l} 0.14 \pm \\ 0.02^a \end{array}$	$\begin{array}{l} \textbf{4.34} \; \pm \\ \textbf{0.94}^{b} \end{array}$
	FR	В	$5.86 \pm 0.13^{\rm b}$	$\begin{array}{l} 0.05 \pm \\ 0.01^a \end{array}$	$0.09 \pm 0.01^{ m b}$	$57.4 \pm 0.9^{\rm b}$	$\begin{array}{l} 5.03 \pm \\ 0.08^{c} \end{array}$	$\begin{array}{l} \textbf{0.13} \pm \\ \textbf{0.00}^{\text{b}} \end{array}$	$\begin{array}{l} \textbf{7.17}  \pm \\ \textbf{0.17}^{\text{a}} \end{array}$	$8.26 \pm 0.$ $46^{b}$	$\begin{array}{l} 0.40 \pm \\ 0.03^{b} \end{array}$	$6.32 \pm \\0.85^a$
	FR	С	$6.07 \pm 0.09^{ab}$	<loq< td=""><td><math display="block">\begin{array}{l} 0.23 \; \pm \\ 0.03^a \end{array}</math></td><td><math display="block">\begin{array}{l} \textbf{58.8} \pm \\ \textbf{0.4}^{ab} \end{array}</math></td><td><math display="block">\begin{array}{l} 5.40 \pm \\ 0.06^{\mathrm{b}} \end{array}</math></td><td><math>0.15 \pm 0.01^{a}</math></td><td><math display="block">\begin{array}{l} 6.79 \pm \\ 0.14^{b} \end{array}</math></td><td><math>8.76 \pm 0.19^{ab}</math></td><td><math display="block">\begin{array}{l} 0.09 \pm \\ 0.01^b \end{array}</math></td><td><math display="block">\begin{array}{l} 5.56 \pm \\ 0.32^{ab} \end{array}</math></td></loq<>	$\begin{array}{l} 0.23 \; \pm \\ 0.03^a \end{array}$	$\begin{array}{l} \textbf{58.8} \pm \\ \textbf{0.4}^{ab} \end{array}$	$\begin{array}{l} 5.40 \pm \\ 0.06^{\mathrm{b}} \end{array}$	$0.15 \pm 0.01^{a}$	$\begin{array}{l} 6.79 \pm \\ 0.14^{b} \end{array}$	$8.76 \pm 0.19^{ab}$	$\begin{array}{l} 0.09 \pm \\ 0.01^b \end{array}$	$\begin{array}{l} 5.56 \pm \\ 0.32^{ab} \end{array}$
11	FR	A	$3.97 \pm 0.14^{\rm b}$	$0.06 \pm 0.00^{a}$	$0.17 \pm 0.01^{a}$	$59.9 \pm 0.9^{a}$	$5.37 \pm 0.03^{a}$	0.09 ± 0.00 <sup>b</sup>	$4.20 \pm 0.15^{a}$	9.64 ± 0.27 <sup>a</sup>	0.09 ± 0.01°	$8.26 \pm 1.64^{a}$
	FR	В	$3.89 \pm 0.04^{\rm b}$	<loq< td=""><td><math>0.16 \pm 0.03^{a}</math></td><td><math display="block">51.9 \pm 0.5^{\rm c}</math></td><td><math>4.27 \pm 0.04^{c}</math></td><td><math display="block">\begin{array}{l} \textbf{0.08} \pm \\ \textbf{0.00}^{\text{c}} \end{array}</math></td><td><math>4.04 \pm 0.04^{a}</math></td><td><math>7.91 \pm 0.09^{ m b}</math></td><td><math display="block">\begin{array}{l} 0.36 \pm \\ 0.07^a \end{array}</math></td><td><math>9.07 \pm 0.60^{a}</math></td></loq<>	$0.16 \pm 0.03^{a}$	$51.9 \pm 0.5^{\rm c}$	$4.27 \pm 0.04^{c}$	$\begin{array}{l} \textbf{0.08} \pm \\ \textbf{0.00}^{\text{c}} \end{array}$	$4.04 \pm 0.04^{a}$	$7.91 \pm 0.09^{ m b}$	$\begin{array}{l} 0.36 \pm \\ 0.07^a \end{array}$	$9.07 \pm 0.60^{a}$
10	FR	C	4.23 ± 0.08 <sup>a</sup>	0.04 ± 0.00 <sup>b</sup>	0.08 ± 0.00 <sup>b</sup>	56.7 ± 0.3 <sup>b</sup>	5.00 ± 0.04 <sup>b</sup>	$0.10 \pm 0.00^{a}$	4.11 ± 0.04 <sup>a</sup>	9.28 ± 0.09 <sup>a</sup>	$0.21 \pm 0.02^{\rm b}$	$8.78 \pm 1.00^{a}$
12	FR	A	$30.2 \pm 0.9^{a}$	$0.10 \pm 0.00^{a}$	0.47 ± 0.05 <sup>a</sup>	85.7 ± 0.7 <sup>a</sup>	$17.9 \pm 0.9^{a}$	$0.31 \pm 0.03^{a}$	$3.41 \pm 0.05^{a}$	$38.0 \pm 2.0^{a}$	0.29 ± 0.04 <sup>b</sup>	$17.9 \pm 3.7^{a}$
	FR	В	$25.7\pm1.7^{\rm b}$	$\begin{array}{l} \textbf{0.08} \pm \\ \textbf{0.00}^{\text{b}} \end{array}$	$0.32 \pm 0.01^{ m b}$	$85.5 \pm 1.4^a$	$18.3\pm1.4^{\mathrm{a}}$	$\begin{array}{l} \textbf{0.27} \pm \\ \textbf{0.02}^{\rm ab} \end{array}$	$3.03 \pm 0.02^{\rm b}$	$32.3\pm2.9^{b}$	$0.29 \pm 0.01^{\rm b}$	$22.4 \pm 2.9^{a}$

(continued on next page)

Table 2 (continued)

Samples	Brands	Lot	Ca (mg/100 g)	Cu (mg/ 100 g)	Fe (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	Mn (mg/100 g)	Na (mg/100 g)	P (mg/100 g)	Zn (mg/100 g)	Se (μg/100 g)
	FR	С	$22.2\pm1.9^{b}$	0.06 ± 0.01°	$0.26 \pm 0.02^{\rm b}$	$83.0 \pm 1.2^{a}$	$13.2\pm0.1^{\rm b}$	$0.25 \pm 0.02^{\rm b}$	$2.77 \pm 0.02^{c}$	$25.8\pm1.6^{\rm c}$	$0.66 \pm 0.05^{a}$	$20.0\pm1.3^{\text{a}}$
13	BT	A	$221\pm3^a$	$\begin{array}{l} 0.14 \pm \\ 0.00^a \end{array}$	$\begin{array}{l} 1.34 \; \pm \\ 0.02^a \end{array}$	$124\pm2^a$	$23.2\pm0.3^{\text{a}}$	$\begin{array}{l} 0.18 \pm \\ 0.00^a \end{array}$	$158\pm4^a$	$178\pm2.^{b}$	$\begin{array}{l} 0.19 \pm \\ 0.03^b \end{array}$	$4.30 \pm 0.87^{a}$
	BT	В	$228\pm12^a$	$0.15 \pm 0.01^{a}$	$1.36 \pm 0.05^{a}$	$128\pm1^a$	$22.6\pm1.3^{a}$	$\begin{array}{l} 0.18 \pm \\ 0.01^a \end{array}$	$157\pm8^a$	$188\pm1^a$	$\begin{array}{l} 0.24 \pm \\ 0.03^{b} \end{array}$	<loq< td=""></loq<>
	BT	С	$181\pm6^{\rm b}$	$\begin{array}{l} 0.13 \pm \\ 0.00^{\mathrm{b}} \end{array}$	$1.00 \pm 0.06^{ m b}$	$94.2 \pm \\ 2.9^{\rm b}$	$16.9\pm0.6^{b}$	$0.16 \pm 0.01^{ m b}$	$116\pm 4^{b}$	$140\pm5^c$	$0.34 \pm 0.02^{a}$	$4.87 \pm 0.26^{a}$
14	MD	A	$\begin{array}{c} \textbf{7.60} \pm \\ \textbf{0.07} \end{array}$	$0.07 \pm 0.00$	$\begin{array}{c} 0.10 \; \pm \\ 0.00 \end{array}$	$134 \pm 0.4$	$10.4 \pm 0.03$	$\begin{array}{c} \textbf{0.20} \pm \\ \textbf{0.00} \end{array}$	$12.7\pm0.1$	$14.1\pm0.1$	$\begin{array}{c} 0.19 \pm \\ 0.01 \end{array}$	$\begin{array}{c} \textbf{7.50} \pm \\ \textbf{0.18} \end{array}$
15	MD	A	$\begin{array}{c} \textbf{2.98} \pm \\ \textbf{0.03} \end{array}$	$0.07 \pm 0.00$	$0.09 \pm 0.00$	$128\pm2$	$\textbf{8.26} \pm \textbf{0.10}$	$\begin{array}{c} \textbf{0.11} \pm\\ \textbf{0.00} \end{array}$	$14.8 \pm 0.2$	$15.2\pm0.2$	$\begin{array}{c} 0.24 \pm \\ 0.03 \end{array}$	$8.66 \pm \\ 0.21$
16	PV	A	$56.9 \pm 0.4$	$0.08 \pm 0.00$	$\begin{array}{c} 0.27 \pm \\ 0.01 \end{array}$	$157\pm1$	$13.5 \pm 0.1$	$\begin{array}{c} \textbf{0.23} \pm \\ \textbf{0.00} \end{array}$	$19.3 \pm 0.2$	$50.4 \pm 0.4$	$\begin{array}{c} 0.29 \pm \\ 0.03 \end{array}$	<loq< td=""></loq<>
17	PV	Α	$85.0\pm1.7$	$0.11\pm0.01$	$0.41 \pm 0.03$	$223\pm 4$	$18.2 \pm 0.3$	$0.32 \pm 0.00$	$18.2\pm0.4$	$71.6\pm1.4$	0.27 ± 0.02	<loq< td=""></loq<>
18	IN	Α	$153\pm 9$	<loq< td=""><td><loq< td=""><td><math display="block">180\pm11</math></td><td><math display="block">13.3 \pm 0.8</math></td><td><loq< td=""><td><math display="block">47.7\pm2.8</math></td><td><math display="block">108\pm7</math></td><td>0.49 ± 0.05</td><td><math display="block">14.2\pm1.5</math></td></loq<></td></loq<></td></loq<>	<loq< td=""><td><math display="block">180\pm11</math></td><td><math display="block">13.3 \pm 0.8</math></td><td><loq< td=""><td><math display="block">47.7\pm2.8</math></td><td><math display="block">108\pm7</math></td><td>0.49 ± 0.05</td><td><math display="block">14.2\pm1.5</math></td></loq<></td></loq<>	$180\pm11$	$13.3 \pm 0.8$	<loq< td=""><td><math display="block">47.7\pm2.8</math></td><td><math display="block">108\pm7</math></td><td>0.49 ± 0.05</td><td><math display="block">14.2\pm1.5</math></td></loq<>	$47.7\pm2.8$	$108\pm7$	0.49 ± 0.05	$14.2\pm1.5$

Mean value  $\pm$  standard deviation (n = 3). Mean with different letters in the same column, for the same sample, indicate a significant difference (p < 0.05) determined using one-way ANOVA and Tukey test at 95% of confidence. LOQ: limit of quantification: 0.4  $\mu$ g/100 g (Se); 0.04  $\mu$ g/100 g (Cu, Fe, Mn and Zn); 0.58  $\mu$ g/100 g (Mg); 1.64  $\mu$ g/100 g (Ca and Na); 2.44  $\mu$ g/100 g (K); 2.48  $\mu$ g/100 g (P). Samples 1, 2, and 3 contain protein isolates (pea protein and non-transgenic soy) as the main ingredient; sample 13 contains cocoa powder and soy protein isolate; samples 4, 5, 6, and 13 are fortified with tricalcium phosphate; and samples 8, 9, 10, 12, 14, and 16 contain fruit in the composition; sample 18 is made with cow's milk, and the others are made with coconut.

a sample of natural yogurt of animal origin were purchased from commercial establishments in the municipality of Campinas (São Paulo, Brazil), totaling 5 brands and 17 different flavors. For each sample, 1–3 distinct lots were purchased. The information on the sample labels is described in our previous study (Rebellato et al., 2023).

### 2.4. Analytical control

Certified reference materials and a plant-based yogurt sample (Sample F, Brand IVV, Lot 1) were used for method validation concerning the figures of merit: accuracy, precision, linearity, the limit of detection, and limit of quantification (AOAC, 2016; INMETRO, 2020).

Linearity was determined using five-point analytical curves for each element. Precision was determined by calculating the coefficient of variation of 7 independent repetitions of the yogurt sample. Method accuracy was evaluated using certified reference materials (CRM), including peach leaves for the elements Mn and Zn, and skimmed milk powder for Ca, Cu, Fe, K, Mg, Na, Se, and P. The limits of detection and quantification were estimated from the analyte concentration corresponding to the mean value of a blank sample plus three and five standard deviations, respectively.

Certified standard solutions were used to construct the analytical curve and were prepared from dilutions of the certified standard solutions, with five points, ranging from 0. 0.41–41.0 mg/100 mL for Ca and Na; 0.062–62.0 mg/100 mL for P; 0.015–14.5 mg/100 mL for Mg; 0.061–61.0 mg/100 mL for K; 0.001–1.0 mg/100 mL for Cu, Fe, Mn, and Zn and 0.01–10.0  $\mu g/100$  mL for Se. Certified standard solutions of Sc, Ge, In, Rh, Bi, and Pt at 1000 mg/L (Fluka, Steinheim, Germany) were used as an internal standard solution at the concentration of 50  $\mu g/L$  to correct for matrix and instrument drift. The analytical control for the inorganic elements considered toxic (Al, Ba, Cr, Co, Mo, and Ni) was reported in a previous study by our research team (Rebellato et al., 2023).

### 2.5. Sample preparation

The samples were mineralized as described by Rebellato et al. (2023). For that, 0.5 g of sample was weighed into a graduated tube (50 mL) and 4 mL of  $\rm HNO_3$  and 2 mL of  $\rm H_2O_2$  were added, and the tube was kept at rest overnight. Then, the tubes were heated in an ultrasonic bath

at 80  $^{\circ}\text{C}$  for 35 min, and cooled down to room temperature, the volume was made up to 20 mL with ultrapure water and filtered with a 0.45  $\mu m$  PTFE filter (Agilent Technologies, Tokyo, Japan). All mineralization procedures were performed in triplicate, including the analytical blank.

### 2.6. Effect of digestion in vitro on the bioaccessibility of inorganic elements of plant-based yogurts

The in vitro digestion was determined according to the INFOGEST 2.0 protocol (Brodkorb et al., 2019), with modifications. Enzyme activities were evaluated before starting the assays,  $\alpha$ -amylase (10,080, 30 U/mg), porcine pepsin (P6887, 3843 U/mg), bovine bile (B3883), pancreatin (P7545, 7.2 U) were used. The other reagents employed to prepare the salivary fluids, gastric and enteric, and to determine enzyme activity followed the protocol specifications. All reagents and enzymes used were from Sigma Chemical Co., St. Louis (USA).

The gastric lipase enzyme was not used due to unavailability, and the protocol was adjusted to use 2.5 g of sample. At the end of the intestinal phase, the samples were centrifuged at 3500 g at 4  $^{\circ}\text{C}$  for 30 min and the upper phase was transferred to digestion tubes, which were then incubated at 100  $^{\circ}\text{C}$  overnight. The mineralization was performed according to the sample preparation as previously described. All analyses were performed in 3 separate repetitions.

### 2.7. Statistical analysis

The results were analyzed by F-test, one-way ANOVA and Tukey's test (p < 0.05) for comparison of means (x $^-\pm$  SD) when necessary, using the software Statistica 7.0 (StatSoft, EUA). The multivariate analysis was conducted by Principal Component Analysis (PCA), and the software Piroutte 3.11 (Infometrix, Inc., Bothell, WA, USA) was used to analyze the results.

### 3. Results and discussion

## 3.1. Analytical method for determining the contents of inorganic elements in plant-based and animal-based yogurts

Linearity was evaluated using five-point analytical curves for each element studied. The analytical curves were linear with  $R^2 > 0.99$  for all

Table 3

Contribution (%) of consuming one serving of plant-based yogurt per day to a recommended daily intake (ANVISA, 2020) of essential inorganic elements.

Individual	Plant-based yogurt										
	Ca	Cu	Fe	Mg	Mn	P	Zn	Se	Na	K	
Adults	11.8	15.1	5.95	3.47	7.37	12.1	5.25	3.16	2.43	3.91	
	0.28 - 40.8	7.56-30.2	0.85 - 18.0	1.51-9.66	2.83-19.3	1.89-46.0	0.62 - 16.4	1.13-6.17	0.24-14.1	1.31-11	
Pregnant	9.10	13.6	3.09	3.65	11.1	6.8	4.82	3.16	2.43	3.91	
	0.21 - 31.4	6.80-27.2	0.44-9.32	1.58-10.1	4.25-28.9	1.125.8	0.57 - 15.0	1.13-6.17	0.24-14.1	1.31-11	
Breastfeeding woman	9.10	10.5	8.33	4.05	8.50	6.76	4.45	2.71	2.43	3.91	
_	0.21 - 31.4	5.23-20.9	1.19-25.2	1.76-11.3	3.27-22.2	1.06-25.8	0.52 - 13.9	0.97-5.29	0.24-14.1	1.31-11	
Children aged 4-8 years	11.8	30.9	8.33	11.2	14.7	16.9	11.6	6.32	2.4	3.91	
	0.28-40.8	15.5-61.8	1.19-25.2	4.86-31.2	5.67-38.5	2.65-64.5	1.36-36.0	2.27-12.3	0.24-14.1	1.31-11	
Individual	Animal-based yogurt										
	Ca	Cu	Fe	Mg	Mn	P	Zn	Se	Na	K	
Adults	26.0	-	-	5.38	-	26.3	7.58	4.03	4.05	8.73	
Pregnant	20.0	-	-	5.65	-	14.7	6.95	4.03	4.05	8.73	
Breastfeeding woman	20.0	-	-	6.27	-	14.7	6.41	3.45	4.05	8.73	
Children aged 4-8 years	26.0	-	-	17.4	-	36.9	16.7	8.06	4.05	8.73	

170 g serving. Contributions are for the average, minimum, and maximum value of the plant-based yogurt.

elements. The tendency/recovery was performed with certified reference material, with percent recovery ranging from 99 (Fe) to 111% (Ca). In addition, all the expanded uncertainties were larger than the difference between mean measured value and certified value, indicating the measured mean value is not significantly different from the certified value.

The precision was determined on 7 repetitions per sample, ranging from 2% to 14%, which met the CV specifications provided by the INMETRO (2020). The limit of detection (LOD) varied from 0.1  $\mu g/100~g$  to 0.8 mg/100 g, and the limit of quantification (LOQ) from 0.4  $\mu g/100~g$  to 2.5 mg/100 g for the elements studied.

### 3.2. Total content of essential inorganic elements in plant-based and animal-based yogurts

The concentration of the inorganic elements of the plant-based and animal-based yogurts is presented in Table 2. Great differences were observed for the samples, including the plant-based yogurts of the same brand and different lots. The analyses were also performed for an animal-based yogurt (sample 18) for comparison purposes between plant-based and animal-based samples. For animal-based yogurt (sample 18), the elements Cu, Fe, and Mn were below the LOQ (0.04 mg/100  $\,$ g). In addition, levels of 14.2  $\mu g/kg$ , and 153, 180, 13.3, 47.7, 108, and 0.49 mg/100 g were observed for the elements Se, Ca, K, Mg, Na, P, and Zn, respectively. Literature data reported values of 143, 0.02, 71, 11, 52, 119, and 0.4 mg/100 g for Ca, Cu, K, Mg, Na, P, and Zn, respectively, for the same type of sample (plain yogurt) (TACO, 2011). Except for K, which had a higher content, the other elements showed similar values to those reported in the literature. Abou Jaoude et al. (2010) analyzed the chemical composition, mineral content, and cholesterol levels of cheese and yogurt of animal origin. The authors found that the contents ranged from 79 to 100; 130-146; 166-237; 13-21; 87-99; and 1-2 mg/100 g for K, P, Na, Mg, Ca, and Zn, respectively, in whole yogurt samples, and the K and Ca contents were lower than those obtained in our study.

For the plant-based samples, the Ca content ranged from <LOQ (1.64) to 228 mg/100 g (sample 13, brand BT, lot C). The highest values were observed for the samples fortified with tricalcium phosphate, as stated on the labels (samples 4, 5, 6, and 13). Of these samples, only sample 13 contained soy protein isolate in its composition, while the others (4, 5, and 6) contained coconut cream as the main ingredient.

Although the samples showed great variation in results when comparing the Ca content of the animal-based yogurt (153 mg/100 g) with the samples not subjected to fortification (14.2 mg/100 g), a reduction of approximately 90% was observed in the Ca content of the plant-based samples. According to the literature, Ca is added to most alternative plant-based milk to mimic the levels present in animal products (134  $\pm$  8.6 mg/100 g) (Vanga & Raghavan, 2018).

Only sample 7 (brand VV) showed results below the LOQ for lots A and B. Another relevant factor is the significant difference (p < 0.05) in Ca content observed in at least one of the lots of the same brand, probably due to processing conditions and the raw material used.

Although the elements Cu, Fe, Mn, and Zn were present in low concentrations in the plant-based yogurts, the values ranged from < 0.04-0.16; 0.07-1.48; 0.05-0.34; and < 0.04-1.06 mg/100 g, respectively, which was not observed in the animal-based yogurt (content < LOQ for Cu, Fe, and Mn), except for Zn, with 0.49 mg/100 g. In addition, only samples 7 (Cu), 9, and 10 (Fe) did not present significant differences among the 3 lots analyzed, which demonstrates the variability of mineral composition among the samples.

The sodium and potassium contents varied from 2.77 to 158, and 27.4–223 mg/100 g, respectively, with no significant differences among the lots for samples 12 (K), 4, and 11 (Na).

When comparing the Na and K levels of the animal-based samples (47.7 and 180 mg/100 g, respectively) with those of plant-based samples, samples 1, 2, 3, and 13 had Na contents higher than 48 mg/100 g. The factor that differentiates these samples from the others is the presence of pea protein isolate and non-transgenic soybeans in samples 1, 2, and 3; and cocoa powder, soy protein isolate, and salt (sodium chloride) in sample 13, while the others contain milk or cream or coconut pulp in their composition. Regarding element K, only sample 9 (lots A and B) containing apricots, and sample 17, which requires reconstitution in water or milk before consumption, showed a content higher than 180 mg/100 g.

Concerning the elements magnesium (Mg) and phosphorus (P), the contents varied from 3.72 to 23.2, and 7.80–188 mg/100 g, respectively. It is worth mentioning that the highest contents for Mg and P were observed in sample 13, which contains cocoa powder and soy protein isolate in its composition. When comparing the results (mg/100 g) of the plant-based samples with those of animal origin (Mg =13.3 and P = 108), the plant-based yogurts showed lower contents for both elements Mg (mean value 8.58) and P (mean value 49.7). In addition, all samples showed a significant difference (p < 0.05) in at least one of the lots of the same brand Table 2.

The Se content ranged from < 4 (LOQ) to 22.4  $\mu g/kg$ , and 11.2  $\mu g/kg$  was the mean value among the plant-based yogurts. The Se content of the animal-based yogurt was approximately 27% higher than the value found for the plant-based yogurt, with values of 14.2 and 11.2  $\mu g/kg$ , respectively.

Recent studies are being published on the subject of plant-based yogurts (Devnani et al., 2022; Greis et al., 2023; Mehta et al., 2023; Part et al., 2023), but work on the content of essential and potentially toxic inorganic elements and their bioaccessibility is still scarce in the literature.

There are no reports on the mineral composition of plant-based

Table 4
Total content (mg/100 g), soluble fraction (mg/100 g), and bioaccessibility percentage of essential elements of plant-based and animal-based yogurts.

Samples	Parameters	Ca	Mg	P	Zn	Mn	Cu	Fe	Se
2	Total content	nd	20.9 ± 0.7	163 ± 6	nd	nd	$0.13 \pm 0.00$	1.50 ± 0.10	
	Soluble fraction % bioaccessible		$1.60 \pm 0.3$ 7.66	$29.5 \pm 0.3$ 18.1			$0.07 \pm 0.00$ 53.9	$0.49 \pm 0.04$ 32.7	nd
5	Total content	$158\pm2$	$3.56 \pm 0.05$	$67.8 \pm 0.53$	nd	nd	nd	nd	
	Soluble fraction	$27.2 \pm 2.6$	$2.08 \pm 0.14$	$51.2 \pm 5.6$					nd
	% bioaccessible	17.2	58.5	75.4					
12	Total content	nd	$16.6\pm1.2$	$34.5\pm1.0$	nd	$0.28\pm0.03$	nd	nd	
	Soluble fraction		$8.27\pm0.45$	$13.1\pm3.0$		$0.08 \pm 0.00$			nd
	% bioaccessible		49.8	38.0		28.6			
13	Total content	$47.2\pm3.1$	$14.6 \pm 0.9$	$188\pm1$	nd	nd	$0.15\pm0.01$	nd	
	Soluble fraction	$16.2\pm0.2$	$8.14\pm0.53$	$87.7 \pm 3.9$			$0.09\pm0.00$		nd
	% bioaccessible	34.4	55.6	46.6			61.2		
16	Total content	$56.9\pm0.4$	$4.78\pm0.10$	nd	nd	$0.23\pm0.00$	$0.08\pm0.00$	$1.20\pm0.03$	
	Soluble fraction	$20.7\pm1.5$	$3.36\pm0.33$			$0.07\pm0.00$	$0.02\pm0.00$	$0.11\pm0.00$	nd
	% bioaccessible	36.4	70.3			30.4	25.0	9.17	
18	Total content	$128\pm2$	$13.7\pm0.3$	$102\pm2$	$0.26\pm0.02$	nd	nd	nd	
	Soluble fraction	$51.8\pm0.5$	$8.35\pm0.23$	$77.9 \pm 3.0$	$0.22\pm0.01$				nd
	% bioaccessible	40.6	61.1	76.2	84.6				

Mean value  $\pm$  standard deviation (n = 3); nd: soluble content in the bioaccessible fraction below the LOQ (limit of quantification): 0.4  $\mu$ g/100 g (Se); 0.04 mg/100 g (Cu, Fe, Mn and Zn); 0.58 mg/100 g (Mg); 1,64 mg/100 g (Ca and Na); 2.44 mg/100 g (K); 2.48 mg/100 g (P).

yogurts, with studies only on the base ingredient used to produce plant-based yogurt. Santos et al. (2014) evaluated the mineral composition of coconut milk by ICP OES, which is one of the main ingredients in obtaining plant-based yogurt, and reported values of 5.8–131; < 0.27–1.56; 0.76–5.52; 212–1781; 14.9–201; < 0.11–3.88; 274–625; 26–341; and < 0.74–3.2 µg/g for Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn, respectively. When comparing the results, although similar results were observed for the Ca, Cu, Mg, and Mn contents, the Fe, K, Na, P, and Zn contents were lower than those found in the present study.

Cunha et al. (2022) evaluated the physical properties, bioactive potential, and inorganic element profile of dairy powders enriched with soybean extract. The authors found contents of  $9.02\pm0.30$ ;  $493\pm56$ ;  $28.7\pm1.1$ ;  $34.7\pm0.58$ ;  $5108\pm608$ ;  $9356\pm1125$ ;  $2992\pm69$ ;  $1602\pm63$ ; and  $11,100\pm826$  for Cu, Fe, Mn, Zn, Ca, K, Mg, Na, and P, respectively, in the soy extract powder. As expected, the results were higher than those found in the present study since the inorganic elements were concentrated in the raw material (soybean extract powder). Gawalko et al. (2009) observed the presence of several trace elements in peas, with contents of 482-1550; 2.96-14.6; 28.6-80; 5804-13310; 1011-1515; 3.78-21.3; 1278-6018; <0.05-1.95; and 17.7-79.6 mg/kg for the elements Ca, Cu, Fe, K, Mg, Mn, P, Se, and Zn, respectively.

### 3.3. Contribution of plant-based yogurt consumption to the RDI of essential elements

Table 3 shows the consumption contributions for one serving (170 g) of plant-based and animal-based yogurt in the daily reference values (DRV) of essential elements for adults, pregnant women, breastfeeding women, and children aged 4–8 years. The RDI values are in accordance with the technical regulation IN 75 of the National Health Surveillance Agency in Brazil (Brasil, 2020) (Table S1, Supplementary Material). Due to the wide variation in the mineral composition of the plant-based yogurts, the contributions are presented as average, minimum, and maximum values.

The elements Ca, P, Zn, Mg, K, and Na are part of the mineral composition of animal products, including yogurt (Fuquay et al., 2011; Pedro et al., 2002), and were also found in the samples of the present study. Furthermore, Se contents were observed in both plant-based and animal-based yogurts.

The maximum Ca contribution for plant-based yogurt proved to be higher than 30% for all individuals evaluated, even higher than the Ca contribution of yogurt of animal origin for adults, pregnant, breastfeeding women, and children aged 4–8 years. However, when evaluating the average value, the contributions were lower, corresponding to

approximately half of the values observed in the animal-based yogurt. This fact should be taken into consideration by individuals who do not consume products of animal origin, such as yogurt, once the Ca contribution may be compromised depending on the brand, the process (fortified or not), and the food base. In addition, it is worth mentioning that these findings are based on the total content of the element, which may be not available for absorption in the body.

The contributions of the elements Cu, Fe, and Mn were significant in the plant-based yogurt samples, which were not observed in the animal-based yogurts, which showed contents below the LOQ. For Cu, the RDI contribution can reach 30% in adults and 61% in children depending on the plant-based yogurt consumed. This fact is relevant since the consumption of different foods throughout the day also contributes to the RDI of Cu and other nutrients, which may lead to an excess of this element in the body.

The contributions in the RDI of the elements Mg, P, Zn, and Se were observed for both yogurts (plant-based and animal-based) and were always higher in the animal-based yogurt when compared to the plant-based yogurt. However, when comparing with the maximum values, the contributions of P and Mn can reach 64% and 38% in children aged 4–8 years, respectively. Although the Se contributions were higher in yogurt of animal origin, the values found in the plant-based yogurts were similar. Concerning the elements Na and K, the average contents were lower in the plant-based samples, despite the higher RDI when compared the maximum Na and K levels.

As previously reported, plant-based yogurt is a novel food category, thus caution in consumption is required since there are little data in the literature on the mineral composition of this type of food.

### 4. Estimation of mineral bioaccessibility in plant-based yogurts

Five plant-based yogurt samples (2, 5, 12, 13, and 16) and one animal-based yogurt sample (18) were selected for the estimation of mineral bioaccessibility. The selection took into consideration different brands and compositions. Table 4 presents the results of the total content, soluble fraction, and bioaccessibility percentage of essential elements of the plant-based and animal-based yogurts. The total contents were only presented for the samples with soluble fractions higher than the LOO.

Although total Ca contents were found in samples 2 and 12, the soluble Ca fraction was below the LOQ (<1.64 mg/100 g) in the bioaccessible fraction, thus the bioaccessibility percentage was not determined. In contrast, samples 5, 13, and 16 had bioaccessible percentages of 17%, 34%, and 36%, respectively. Although sample 5 had a high total

Table 5 Mean, minimum, and maximum contents ( $\mu g/kg$ ) of trace elements of plant-based yogurts.

Value	Al	Ba	Co	Cr	Mo	Ni
Mean Minimum	870 < 200	261 < 4	11.7	25.2 < 4	105 < 4	108 < 4
Maximum	9019	1505	40.6	88.1	348	700

Ca content, due to the fortification with tricalcium phosphate, the bioaccessible fraction did not show the same behavior, which contributed to a lower bioaccessibility percentage. Such behavior was not observed for sample 13, once although it is a Ca-fortified sample, it showed a bioaccessibility similar to sample 16, which was not fortified. When comparing the bioaccessibility results of the plant-based yogurt and the animal-based yogurt (40.6%), the bioaccessibility can be reduced by 4–27% depending on the composition of the plant-based sample.

The bioaccessibility percentage of Mg ranged from 7.66% to 70.3% in the plant-based yogurts and was higher when compared to the animal-based yogurt (61.1%). Sample 16 exhibited the highest bioaccessibility percentage (70.3%), followed by sample 5 (58.5%). It is worth noting that both samples contain fruits (strawberry and red fruits, respectively) in their composition, which may have contributed to the higher percentage of bioaccessible Mg.

Concerning element P, the percentages ranged from not detected (sample 16) to 76.2% (sample 18). Sample 5 exhibited 75.4% of bioaccessibility, similar to that of animal origin (76.2%). Regarding Zn, only the animal-based yogurt (18) showed a bioaccessibility percentage (85%), while all plant-based yogurts showed values below LOQ.

About the % of bioaccessible fraction of the elements Mn, Cu, and Fe, only sample 16 presented bioaccessibility for these elements, with percentages of 30.4%, 25.0%, and 9.17%, respectively. The other samples exhibited varied bioaccessibility, with values of 28.6% for Mn (sample 12), 53.9%, and 61.2% for Cu (samples 2 and 13) and 32.7% for Fe (sample 2). It is worth noting that only sample 16 contained red fruits in its composition (blackberry, raspberry, and strawberry), besides requiring reconstitution before consumption (powdered sample). Regarding the element Se, although it was possible to quantify the total content in the samples studied, it was not possible to determine the soluble fraction, which impaired the calculation of % bioaccessibility.

### 4.1. Total content of trace elements and estimated bioaccessibility in plant-based and animal-based yogurts

The total contents of trace and potentially toxic elements in plant-based and animal-based yogurt samples were addressed in detail in a previous study by our research group (Rebellato et al., 2023). As already reported, because it is a new food category, there are few studies on plant-based products, mostly contemplating plant-based beverages. Thus, information about the bioaccessible percentage of trace and potentially toxic inorganic elements in plant-based yogurts has not been found in the literature. Table 5 presents the total contents (average, minimum, and maximum) of the elements selected for the evaluation of bioaccessibility assessment in plant-based yogurt samples.

Table 5 shows a large variation in the composition of the trace elements evaluated. An increase of 10x and 7x was observed for the Al and Ni contents when compared to the maximum levels. However, when comparing the minimum and maximum contents, the increase was 45x, 704x, 175x, 87x, 376x, and 3x for Al, Cr, Ni, Mo, Ba, and Co, respectively. Zhang et al. (2019) evaluated heavy metals in soybeans and found different concentrations of the elements, such as the differences between the minimum and maximum Ni content from 0.53 to 15.0 mg/kg, representing an increase of 28x. Zhang et al. (2021) analyzed the concentration of metals in soybeans from different soil types and reported contents ranging from 0.062 to 0.83 mg Cr/kg, 9.5–15 mg Cu/kg, 0.34–1.1 mg Ni/kg, and 31–120 mg Zn/kg. Gawalko et al. (2009)

Table 6 Total content ( $\mu g/kg$ ), soluble fraction ( $\mu g/kg$ ), and bioaccessible percentage of essential elements of plant-based and animal-based yogurts.

Samples	Parameters	Al	Ba	Co	Cr	Mo	Ni
2	Total content	nd	1000	41.1	41.4	329	277
			$\pm~190$	$\pm$ 7.1	$\pm$ 2.7	$\pm~19$	$\pm 19$
	Soluble		117	4.50	3.23	256	19.4
	fraction		$\pm$ 11	$\pm~0.2$	$\pm~0.3$	$\pm$ 5	$\pm$ 2.5
	%		11.7	10.9	7.81	77.9	7.0
	bioaccessible						
5	Total content	6716	665	nd	74.9	7.31	41.2
		$\pm \ 740$	$\pm$ 59		$\pm$ 6.9	$\pm 0.4$	$\pm$ 6.3
	Soluble	747	360		3.0	6.43	37.0
	fraction	$\pm$ 62	$\pm$ 2		$\pm 1.3$	$\pm~0.4$	$\pm$ 2.5
	%	11	54.1		48.0	87.9	89.9
	bioaccessible						
12	Total content	nd	1085	nd	nd	nd	106
			$\pm~60$				$\pm 1$
	Soluble		328				37.1
	fraction		$\pm 6$				$\pm 1.1$
	%		30.2				35.0
	bioaccessible						
13	Total content	nd	700	28.0	37.0	nd	454
			$\pm$ 24	$\pm$ 2.3	$\pm$ 3.2		$\pm 34$
	Soluble		237	25.0	6.22		136
	fraction		$\pm~20$	$\pm \ 1.2$	$\pm~0.2$		$\pm$ 4
	%		33.9	89.0	16.8		29.9
	bioaccessible						
16	Total content	nd	136	nd	30.7	nd	491
			$\pm 31$		$\pm~0.9$		$\pm$ 40
	Soluble		104		19.6		252
	fraction		$\pm 14$		$\pm~0.7$		$\pm 7$
	%		76.1		63.9		51.3
	bioaccessible						
18	Total content	nd	nd	nd	nd	58.5	nd
						$\pm~1.2$	
	Soluble					41.5	
	fraction					± 3.9	
	%					70.9	
	bioaccessible						

Mean value  $\pm$  standard deviation (n = 3); nd: soluble content in the bioaccessible fraction below the LOQ (limit of quantification): 4 µg/kg (Ba, Co, Cr, Mo and Ni); 200 µg/kg (Al).

determined the levels of various trace elements in peas to ensure the safety of the grain and reported levels of <0.05–0.99 mg Co/kg, <0.025–0.75 mg Cr/kg, and 0.49–8.08 mg Ni/kg.

Although the samples of this study presented low contents of these elements, the major concern associated with toxic inorganic elements is the cumulative toxicity, and non-carcinogenic and/or carcinogenic risk, causing harmful impacts on human health (Kong et al., 2020). In this sense, the knowledge of the total content, as well as the soluble fraction is of great relevance to estimating the availability of absorption of these elements in the body.

The results of the total content, soluble fraction, and bioaccessible percentage of the elements Al, Ba, Co, Cr, Mo, and Ni in plant-based and animal-based yogurts are presented in Table 6.

As can be seen in Table 6, only sample 5 showed bioaccessible Al (%), while the others had levels below the LOQ (200 ppb), including the animal-based yogurt.

Regarding the elements Cr and Mo, daily reference values (DRV) are established for these elements (Table A, Supplementary Material). When calculating the RDI contribution for these 2 elements for the consumption of a 170 g serving of yogurt, values above 120% are observed for adult individuals, pregnant women, breastfeeding women, and children aged 4–8 years for molybdenum. In turn, for chromium, contributions of around 40% were observed for adults, pregnant women, breastfeeding women, and 100% for children aged 4–8 years. Furthermore, when evaluating the bioaccessibility, percentages of 8–64% were obtained for Cr, and above 70% for Mo, reinforcing the recommendation of moderate consumption of this food category.

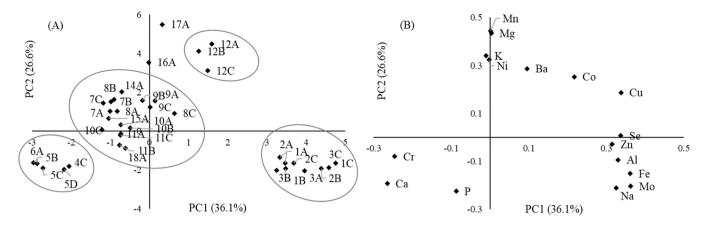


Fig. 1. Principal Component Analysis of the essential and toxic inorganic elements in plant-based and animal-based yogurts (18 A). Graphs of scores (A) and loadings (B).

Concerning the trace elements Ni, Ba, and Co, only the animal-based yogurt did not present quantifiable bioaccessible contents. In turn, the plant-based yogurts showed bioaccessibility percentages of 7–90% for Ni and 12–76% for Ba, and only samples 2 (%) and 13 (89%) showed Co bioaccessibility, with percentages of 11% and 89%, respectively.

#### 5. Principal component analysis (PCA)

Principal Component Analysis (PCA) was performed to check for possible correlations between the essential inorganic and the potentially toxic elements. The PCA was constructed with 45 samples (plant-based and animal-based yogurts) and 16 variables (for the essential and toxic inorganic elements), resulting in a 45  $\times$  16-dimension matrix representing 720 trials. Data were auto-scaled and sample 13 (lots A, B, and C) was considered an outlier. A new PCA was constructed with 42 samples and 16 variables, with a 62.4% variance between PC1 and PC2.

The score plot in Fig. 1A presents the sample distribution, with the formation of 4 distinct groups. The first group was formed by samples 1, 2, and 3 (lots A, B, and C), all from the same brand, and similar ingredient characteristics. The second group corresponded to sample 12, the only one containing red fruits in its composition. Samples 4 C, 5B, 5 C, 5D, and 6 A formed the third group, corresponding to the samples of the same brand, with similar composition characteristics. Finally, the fourth group was formed from the remaining samples, with varied brands and lots, including animal-based yogurt. Samples 16 A and 17B did not correlate with any other group.

The loading plot (Fig. 1B) indicates the effect of the analytes that led to the separation of the samples. The first group contained pea and soy protein as the main ingredient and was grouped due to the higher Al, Fe, Mo, and Na contents. The second group was composed of only one sample, which was classified as having higher Ba and Co contents. The third group, composed of the same brand with different samples and lots, had higher Ca and Cr contents, as declared on the label since they were fortified with Ca. The fourth group, composed of the other samples, including sample 18 A (animal-based yogurt), was probably classified due to the low amounts of all elements. Samples 16 A and 17 A were not grouped because they contained high levels of Ni and K, and Mg and Mn, respectively.

### 6. Conclusion

Different contents of essential inorganic elements were observed in the plant-based yogurts, including samples of the same brand and different lots. Furthermore, the mineral composition of the animal-based yogurt was different from the plant-based sample. These differences were confirmed when comparing the contribution of the consumption of a serving of plant-based or animal-based yogurt per day for the

recommended daily intake.

The exploratory data analysis (PCA) allowed the classification of the samples into distinct groups based on their ingredients, including the samples containing pea and soy protein, which had higher Al, Fe, Mo, and Na contents, and the samples fortified with minerals such as calcium.

The INFOGEST 2.0 protocol was used to estimate the bioaccessibility of essential elements in plant-based yogurts, and the results showed great variation, probably due to the difference in composition among the samples. Concerning the trace elements, although low levels were observed, the bioaccessibility percentage was higher than 50% for some elements, which shows the need for caution in consumption.

Plant-based yogurts are considered a new category of food product, thus data in the literature are still scarce on this subject. In this context, the present study can contribute with data about the composition and the estimated absorption of essential and potentially toxic inorganic elements in plant-based yogurts, as well as the contribution to daily intake, thus ensuring the safety and health of consumers.

### CRediT authorship contribution statement

Conceptualization, A.P.R. and M.A.M.; methodology, A.P.R., M.I.A. F. and R.F.M.; validation, A.P.R., M.I.A.F. and R.F.M.; formal analysis and investigation, A.P.R., M.I.A.F. and R.F.M.; data curation, A.P.R., M.I. A.F. and R.F.M.; writing-original draft preparation, A.P.R.; writing-review and editing, A.P.R., M.I.A.F., R.F.M. and M.A.M.; supervision and funding acquisition, M.A.M. All authors have read and agreed to the published version of the manuscript.

### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### **Data Availability**

Data will be made available on request.

### Acknowledgments

The authors thank the financial support of the São Paulo Research Foundation (FAPESP) (2022/07015-2 and 2017/50349-0) and CNPq (407080/2021-0 and 306054/2020-5).

#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105639.

#### References

- Abou Jaoude, D., Olabi, A., Najm, N.E.O., Malek, A., Saadeh, C., Baydoun, E., Toufeili, I., 2010. Chemical composition, mineral content and cholesterol levels of some regular and reduced-fat white brined cheeses and strained yogurt (Labneh). Dairy Sci. Technol. 90 (6), 699–706. https://doi.org/10.1051/dst/2010026.
- AOAC. (2016). International, Official Methods of Analysis of AOAC International, in Guidelines for Standard Method Performance Requirements (Appendix F. AOAC International
- Bernat, N., Cháfer, M., Chiralt, A., González-Martínez, C., 2014. Vegetable milks and their fermented derivative products. Int. J. Food Stud. 3 (1) https://doi.org/ 10.7455/ijfs/3.1.2014.a9
- Brasil. (2020). INSTRUÇÃO NORMATIVA IN N° 75, DE 8 DE OUTUBRO DE 2020. Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Recio, I., 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat. Protoc. 14 (4), 991–1014. https://doi.org/10.1038/s41596-018-0119.1
- Cardoso, C., Afonso, C., Lourenço, H., Costa, S., Nunes, M.L., 2015. Bioaccessibility assessment methodologies and their consequences for the risk-benefit evaluation of food. Trends Food Sci. Technol. 41 (1), 5–23. https://doi.org/10.1016/j. tifs.2014.08.008.
- CFR, T. 21 of the. (2023). Food and Drugs, Regulations, Code of Federal. https://doi.org/ https://www.ecfr.gov/current/title-21.
- Codina-Torrella, I., Guamis, B., Ferragut, V., Trujillo, A.J., 2017. Potential application of ultra-high pressure homogenization in the physico-chemical stabilization of tiger nuts' milk beverage. Innov. Food Sci. Emerg. Technol. 40, 42–51. https://doi.org/ 10.1016/j.ifset.2016.06.023.
- Cunha, T.M.P., da, Haas, I.C., da, S., da Costa, M.A.J.L., Luna, A.S., de Gois, J.S., Amboni, R.D., de, M.C., Prudencio, E.S., 2022. Dairy powder enriched with a soy extract (Glycine max): physicochemical and polyphenolic characteristics, physical and rehydration properties and multielement composition. Food Res. Int. 162, 112144 https://doi.org/10.1016/j.foodres.2022.112144.
- Devnani, B., Ong, L., Kentish, S.E., Scales, P.J., Gras, S.L., 2022. Physicochemical and rheological properties of commercial almond-based yoghurt alternatives to dairy and soy yoghurts. Future Foods 6, 100185. https://doi.org/10.1016/j.fufo.2022.100185.
- European Commission. COMMISSION REGULATION (EU) 2023/915, on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, 55 (2023). (https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:320 23R0015)
- Felberg, I., Antoniassi, R., Deliza, R., Freitas, S.C. de, Modesta, Della, R.C., 2009. Soy and Brazil nut beverage: processing, composition, sensory, and color evaluation. Ciência e Tecnol. De. Aliment. 29 (3), 609–617. https://doi.org/10.1590/S0101-20612009000300024.
- Fernandez-Garcia, E., Carvajal-Lerida, I., Perez-Galvez, A., 2009. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutr. Res. 29 (11), 751–760. https://doi.org/10.1016/j.nutres.2009.09.016.
- Fioravanti, M.I.A., Rebellato, A.P., Milani, R.F., Morgano, M.A., Bragotto, A.P.A., 2023. Toxic inorganic elements in plant-based beverages: Total concentration, dietary exposure and bioaccessibility. J. Food Compos. Anal. 123, 105565 https://doi.org/ 10.1016/j.jfca.2023.105565.
- Fuquay, J.W., & Fox, P.F., & McSweeney, P.L.H. (2011). Encyclopedia of Dairy Sciences ( & C. B. (Eds. (S. Relton, E. Collins (ed.); 2nd ed). Elsevier Ltd.
- Gawalko, E., Garrett, R.G., Warkentin, T., Wang, N., Richter, A., 2009. Trace elements in Canadian field peas: a grain safety assurance perspective. Food Addit. Contam.: Part A 26 (7), 1002–1012. https://doi.org/10.1080/02652030902894389.
- Greis, M., Nolden, A.A., Kinchla, A.J., Puputti, S., Seppä, L., Sandell, M., 2023. What if plant-based yogurts were like dairy yogurts? Texture perception and liking of plantbased yogurts among US and Finnish consumers. Food Qual. Prefer. 107, 104848 https://doi.org/10.1016/j.foodqual.2023.104848.
- INMETRO. (2020). The National Institute of Metrology, Stardardization and Industrial Quality, DOQ-CGCRE-008, Revision 09 (p. 28).
- Jeske, S., Zannini, E., Arendt, E.K., 2017. Evaluation of physicochemical and glycaemic properties of commercial plant-based milk substitutes. Plant Foods Hum. Nutr. 72 (1), 26–33. https://doi.org/10.1007/s11130-016-0583-0.
- Kong, D., Li, X., Yao, J., He, Y., Luo, J., Yang, M., 2020. Health risk assessment and bioaccessibility of toxic elements in edible and medicinal plants under different consumption methods. Microchem. J. 159, 105577 https://doi.org/10.1016/j. microc.2020.105577.

- Laparra, J.M., Vélez, D., Montoro, R., Barberá, R., Farré, R., 2003. Estimation of arsenic bioaccessibility in edible seaweed by an in vitro digestion method. J. Agric. Food Chem. 51 (20), 6080–6085. https://doi.org/10.1021/jf034537i.
- Lomer, M.C.E., Parkes, G.C., Sanderson, J.D., 2007. Review article: lactose intolerance in clinical practice - myths and realities. Aliment. Pharmacol. Ther. 27 (2), 93–103. https://doi.org/10.1111/j.1365-2036.2007.03557.x.
- Mäkinen, O.E., Wanhalinna, V., Zannini, E., Arendt, E.K., 2016. Foods for special dietary needs: non-dairy plant-based milk substitutes and fermented dairy-type products. Crit. Rev. Food Sci. Nutr. 56 (3), 339–349. https://doi.org/10.1080/ 10408398.2012.761950.
- Mehta, A., Kumar, L., Serventi, L., Schlich, P., Torrico, D.D., 2023. Exploring the textural dynamics of dairy and plant-based yoghurts: a comprehensive study. Food Res. Int. 171, 113058 https://doi.org/10.1016/j.foodres.2023.113058.
- Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Brodkorb, A., 2014. A standardised static in vitro digestion method suitable for food an international consensus. Food Funct. 5 (6), 1113–1124. https://doi.org/10.1039/c3fo60702j.
- Nilva Aparecida Ressinetti Pedro, De, O.E., Penazzi, F.S., Porffrio, Darilena Monteiro, 2002. Estudo do conteúdo mineral de iogurtes naturais e com sabor de frutas, comercializados na cidade de São Paulo, Brasil. Arch. Latinoam. De. Nutr. 51 (2), 210-215.
- European Commission. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, 213 (2023). https://doi.org/http://data.europa.eu/eli/reg/2011/1169/ oi.
- Part, N., Kazantseva, J., Rosenvald, S., Kallastu, A., Vaikma, H., Kriščiunaite, T., Pismennoi, D., Viiard, E., 2023. Microbiological, chemical, and sensorial characterisation of commercially available plant-based yoghurt alternatives. Future Foods 7, 100212. https://doi.org/10.1016/j.fufo.2022.100212.
- Patwa, J., Flora, S.J.S., 2020. Heavy metal-induced cerebral small vessel disease: insights into molecular mechanisms and possible reversal strategies. Int. J. Mol. Sci. 21 (11), 3862. https://doi.org/10.3390/ijms21113862.
- Patwa, J., Sharma, A., Flora, S.J.S., 2022. Arsenic, cadmium, and lead. Reproductive and Developmental Toxicology. Elsevier, pp. 547–571. https://doi.org/10.1016/B978-0-323-89773-0.00029-1.
- Rebellato, A.P., Fioravanti, M.I.A., Milani, R.F., Morgano, M.A., 2023. Inorganic contaminants in plant-based yogurts commercialized in Brazil. Int. J. Environ. Res. Public Health 20 (4), 3707. https://doi.org/10.3390/ijerph20043707.
- Santos, D.C.M.B., Carvalho, L.S.B., Lima, D.C., Leão, D.J., Teixeira, L.S.G., Korn, M.G.A., 2014. Determination of micronutrient minerals in coconut milk by ICP OES after ultrasound-assisted extraction procedure. J. Food Compos. Anal. 34 (1), 75–80. https://doi.org/10.1016/j.jfca.2014.02.008.
- Sethi, S., Tyagi, S.K., Anurag, R.K., 2016. Plant-based milk alternatives an emerging segment of functional beverages: a review. J. Food Sci. Technol. 53 (9), 3408–3423. https://doi.org/10.1007/s13197-016-2328-3.
- Silva, J.G.S., Rebellato, A.P., Caramés, E.T., dos, S., Greiner, R., Pallone, J.A.L., 2020. In vitro digestion effect on mineral bioaccessibility and antioxidant bioactive compounds of plant-based beverages. Food Res. Int. 130, 108993 https://doi.org/10.1016/j.fpodres.2020.108993.
- Singhal, S., Baker, R.D., Baker, S.S., 2017. A Comparison of the Nutritional Value of Cow's Milk and Nondairy Beverages. J. Pediatr. Gastroenterol. Nutr. 64 (5), 799–805. https://doi.org/10.1097/MPG.00000000001380.
- TACO. (2011). Tabela brasileira de composição de alimentos. 4. ed. rev(UNIVERSIDADE ESTADUAL DE CAMPINAS-UNICAMP), 161.
- Tangyu, M., Muller, J., Bolten, C.J., Wittmann, C., 2019. Fermentation of plant-based milk alternatives for improved flavour and nutritional value. Appl. Microbiol. Biotechnol. 103 (23–24), 9263–9275. https://doi.org/10.1007/s00253-019-10175-0
- Thakur, N., Raigond, P., Singh, Y., Mishra, T., Singh, B., Lal, M.K., Dutt, S., 2020. Recent updates on bioaccessibility of phytonutrients. Trends Food Sci. Technol. 97, 366–380. https://doi.org/10.1016/j.tifs.2020.01.019.
- Vanga, S.K., Raghavan, V., 2018. How well do plant based alternatives fare nutritionally compared to cow's milk? J. Food Sci. Technol. 55 (1), 10–20. https://doi.org/ 10.1007/s13197-017-2915-y.
- Xia, Q., Tao, H., Huang, P., Wang, L., Mei, J., Li, Y., 2017. Minerals in vitro bioaccessibility and changes in textural and structural characteristics of uncooked pre-germinated brown rice influenced by ultra-high pressure. Food Control 71, 336–345. https://doi.org/10.1016/j.foodcont.2016.07.018.
- Zhang, S., Song, J., Wu, L., Chen, Z., 2021. Worldwide cadmium accumulation in soybean grains and feasibility of food production on contaminated calcareous soils. Environ. Pollut. 269, 116153 https://doi.org/10.1016/j.envpol.2020.116153.
- Zhang, T., Xu, W., Lin, X., Yan, H., Ma, M., He, Z., 2019. Assessment of heavy metals pollution of soybean grains in North Anhui of China. Sci. Total Environ. 646, 914–922. https://doi.org/10.1016/j.scitotenv.2018.07.335.